REMARKS

Information Disclosure Statement

In the outstanding Office Action under the heading ***Information Disclosure Statement***, it states "[t]here are not entries for the IDS, dated 1/4/07." Applicants assume that that the Examiner is referring to an IDS submission (see Exhibit A), which was attached to the outstanding Office Action, with a slash mark across the page along with a notation, "IDS IS EMPTY," at the top left corner. This form was originally submitted with an IDS dated November 3, 2006. Applicants respectfully point out that in this form there is, in fact, an entry under NON PATENT LITERATURE DOCUMENTS as follows: "English translation of International Preliminary Report on Patentability for PCT/JP2005/001574." This document indicated in the form was indeed provided with the IDS (verified by PAIR). Applicants thus request that the IDS be fully considered as submitted and proper annotations be affixed accordingly in the IDS form.

Pending Claims

Claims 1, 3-5, 8, and 10-16 have been examined. Claims 2, 6, 7, and 9 have been previously canceled. Claims 1, 5, 8, 10-16 have been cosmetically amended. No new matter has been added to any of the amended claims.

Claim Objections

Claim 15 has been objected to for allegedly not having a period at the end of the sentence. However, it appears that this objection should have been directed to claim 16 instead of claim 15. A correction was made to obviate this objection.

The Examiner further requested that Applicants "ascertain that the specification is free of grammatical and typographical errors." Although Applicants will make every reasonable efforts to insure that the specification is free of grammatical and typographical errors, Applicants cannot "ascertain" that it is free of those errors.

Claim Rejections - 35 USC §112

a) Claims 10, 11, and 13-16 have been rejected under 35 USC §112, first paragraph, because the specification allegedly "does not reasonably provide enablement for the inhibition or prevention of all diseases associated with peptidylarginine deiminases or particularly the prevention of MS or RA or psoriasis by said compounds of formula (II*) or salts thereof, as suggested by the breath of the instant claims."

Applicants traverse this indefinite rejection for at least the following reasons. Applicants respectfully point out that the claims 10 and 11 depend directly or indirectly from claim 8 and are directed to the peptidylarginine deiminase 4 inhibitor. That is, the claims are directed to the compound itself. Claims 10 and 11, as amended, do not claim that the compound inhibit or prevent diseases but claim that it is "provided to inhibit enzymatic activities of peptidylarginine deiminases 4." For at least these reasons, claims 10 and 11 are fully enabled for what they claim.

Claims 13 and 15 have been amended to recite a step: "administering to a subject the peptidylarginine deiminiase 4 inhibitor to inhibit the enzymatic activities of peptidylarginine deiminase 4." Therefore, these claims are fully enabled by the present

application because the specification would have shown to a person of ordinary skill in the art that the inhibitor does inhibit peptidylarginine deiminase 4.

Claims 14 and 16 have been amended to recite, "one or more diseases associated with peptidylarginine deiminase 4 and suffered by the subject are selected from the group consisting one or more of rheumatoid arthritis, psoriasis, and multiple sclerosis," to claim that there are connections between peptidylarginine deiminase 4 and the diseases and to denote that inhibition of peptidylarginine deiminase 4 would have an impact on the diseases. Support for this can be found, for example, on page 2, line 29 to page 3, line 7 of the present specification:

> Recently, it has been reported that the presence of a single nucleotide polymorphism (SNP) in the PAD4 [peptidylarginine deiminase 4] gene suppresses the mRNA decay to produce excess citrullinated proteins and thereby autoantibodies against the citrullinated proteins are formed in the blood of rheumatoid arthritis patients. This suggests that PAD4 is strongly involved in the development of rheumatoid arthritis (non-patent document 24).[1]

Claims 14 and 16 are fully supported by the present application.

With these amendments, Applicants submit that the 112 rejections have been obviated.

b) Claims 1, 3, 4, 5, 8, and 10-12 have been rejected under 35 USC §112, second paragraph, as being allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically,

---

[1] Non-patent document 24: Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y. and Yamamoto, K. (2003) *Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis.* Nature Genetics, 34, 395-402. (Page 5, line 7 from the bottom to page 6, line 2 from the top.)

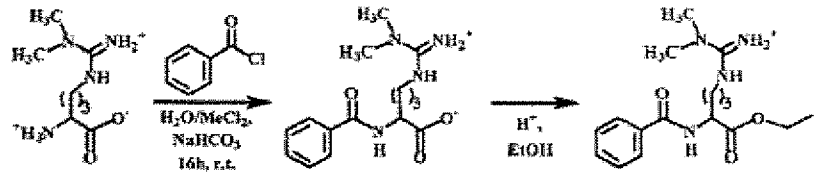
the claims have been rejected for using Formula (II), (Ia), (Ib), (Ic), and (II') on one hand and also using Formula (1), (2), (3), (4), and (5) on the other. To obviate this rejection, Applicants have deleted all references to Formula (1), (2), (3), (4), and (5) to clarify the nomenclature. Withdrawal of this rejection is respectfully requested.

c) Claim 12 has been rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, claim 12 has been rejected for allegedly missing essential manufacturing steps. Claim 12 has been amended to recite as a compound claim. Therefore, Applicants believe that this rejection has been obviated.

Claim Rejections - 35 USC §102

Claims 1, 3, 4, 5, 8 and 10-12 have been rejected under 35 USC §102(b) as being anticipated by Keaney et al. *Kinetic Characterization of Protein Arginine Deiminase 4; A transcriptional Corepressor Implicated in the Onset of Progression of Rheumatoid Arthritis.* Biochemistry (2005), 44(31), 10570-10582. Particularly, the Examiner states that "Kearney et al. teach the synthesis of dimethyl-benzoyl-L-arginine ethyl ester in Scheme I, page 10578." Scheme I is reproduced below.

Scheme 1: Synthesis of Asymmetric Dimethylbenzoyl-L-arginine Ethyl Ester (6b)



The Examiner elaborates that Kearney et al. anticipates the instant claims when not all

R^1, R^2, R^3 are all H; or alternatively, "provided that at least one of R^1, R^2, R^3 does not represent H"; R^4= substituted N and R^5= substituted -COOH.

However, Applicants respectfully submit that Kearney et al. is not prior art to the present application. Keaney et al. was published sometime in 2005. The present application, on the other hand, is a national phase application of PCT/JP05/01574 filed on February 3, 2005, which claims priority to Japanese Application No. 2004-028467 filed on February 4, 2004. Therefore, the priority filing date of the present application antedates the publication of Kearney et al. Accordingly, Kearney et al. is not prior art to the present application. An English translation of the certified copy of the Japanese application is attached as Exhibit B. Withdrawal of this rejection is respectfully requested.

Summary

For the foregoing reasons, the present application is in condition for allowance.

Please charge any required fees for the filing of this Amendment to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: 9/25/08

By: _____
Chris T. Mizumoto
Reg. No. 42,899

Enclosures

EXHIBIT A

IDS IS EMPTY!

/ML/ 03/19/2008

Substitute for form 1449A/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				*Application Number*	10/588,451
				Filing Date	August 4, 2006
				First Named Inventor	Mamoru SATO
(Use as many sheets as necessary)				*Art Unit*	1752
				Examiner Name	Not Known
Sheet	1	of	1	*Attorney Docket Number*	10084.0017

U.S. PATENTS AND PUBLISHED U.S. PATENT APPLICATIONS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] *(if known)*	Issue or Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		US-			
		US-			
		US-			
		US-			
		US-			
		US-			

Note: Copies of the U.S. Patent Documents are not Required in IDS filed after October 21, 2004

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3] Number[4] Kind Code[5] *(if known)*	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	Translation[6]

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	Translation[6]
		English translation of International Preliminary Report on Patentability for PCT/JP2005/001574	

Examiner Signature	/Marialouisa Lao/	Date Considered	01/04/2008

EXHIBIT B

VERIFICATION OF TRANSLATION

Sir:

Setsuko Mayama, a translator, residing at NOMURA & MAYAMA, c/o 30-1, Tsuruyacho 3-chome Kanagawa-ku, Yokohama-shi, Kanagawa 221-0835 Japan

Hereby states:

(1) that I know well both the Japanese and English languages;

(2) that the attached English translation is a true and correct translation of Japanese Patent Application No. 2004-028467 filed on February 4, 2004 made by me to the best of my knowledge and belief.

Date: September 22, 2008 By: _____

Setsuko Mayama

(Translation)

JAPAN PATENT OFFICE

This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: February 4, 2004

Application Number: Japanese Patent Application
No. 2004-028467
[JP2004-028467]

Applicant(s): Yokohama City

March 10, 2005

Commissioner,
Japan Patent Office Hiroshi OGAWA (seal)

Certificate No. 2005-3020411

[Name of document] Patent Application

[Docket Number] P03-073

[Filing Date] February 4, 2004

[Addressee] Commissioner of the Patent Office

[IPC] C07C

A01K

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[Indication of Fees]

[Deposit Ledger No.] 093194

[Amount Paid] 21,000 Yen

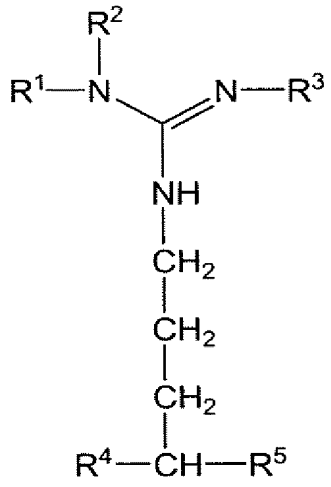
[List of Attached Documents]

[Name of Document]	Claims	1
[Name of Document]	Specification	1
[Name of Document]	Drawing	1
[Name of Document]	Abstract	1

[Name of Document] Claims

[Claim 1] A compound represented by the general formula (I) or a salt thereof:

[Formula 1]

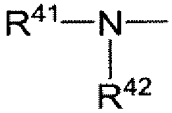


(I)

wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or an alkyl group having 1 to 3 carbon atoms, provided that at least one of R^1, R^2 and R^3 does not represent a hydrogen atom; R^4 represents an amino group which has a substituent; and R^5 represents a carboxyl group which may have a substituent.

[Claim 2] The compound or salt thereof according to Claim 1, wherein R^4 represents the following formula:

[Formula 2]



wherein R^{41} represents a group represented by $R^{401}CO-$ where R^{401}

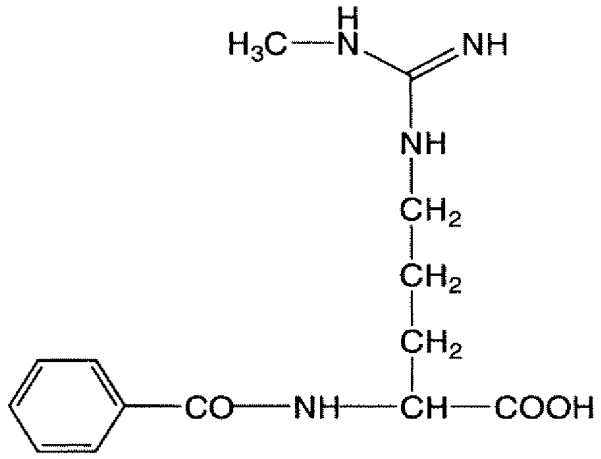
represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, a group represented by $R^{402}S(O)_m$- where R^{402} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and m is an integer of 1 or 2, or a group represented by $R^{405}N(R^{406})$-CHR^{404}-CO-$[NH-CHR^{403}-CO]_n$- where R^{403}, R^{404}, R^{405} and R^{406} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and n is an integer of 1 to 50; and R^{42} represents a hydrogen atom or an alkyl group having 1 to 3 carbon atoms.

[Claim 3] The compound or salt thereof according to Claim 2, wherein R^{41} represents a benzoyl group which may have a substituent, a benzoylpeptidyl group which may have a substituent, a dansyl group which may have a substituent or a dansylpeptidyl group which may have a substituent; and R^{42} represents a hydrogen atom.

[Claim 4] The compound or salt thereof according to any one of Claims 1 to 3, wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or a methyl group, provided that at least one of R^1, R^2 and R^3 represents a methyl group.

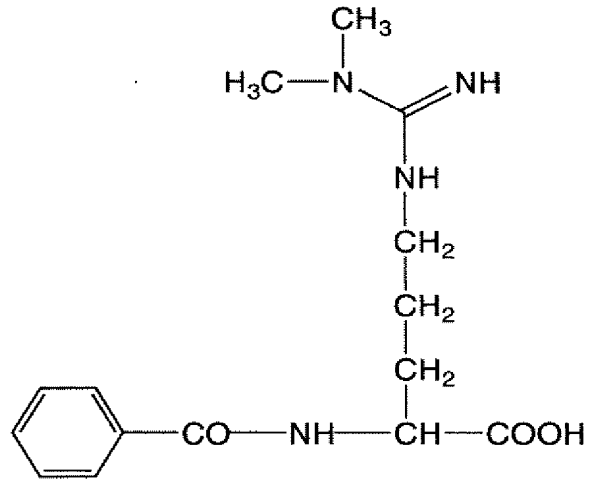
[Claim 5] The compound or salt thereof according to Claim 4, which is a compound represented by the formula (Ia), (Ib) or (Ic) or a salt thereof.

[Formula 3]



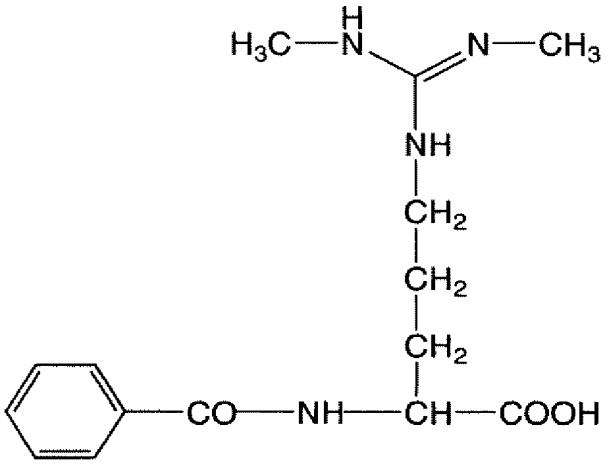
(Ia)

[Formula 4]



(Ib)

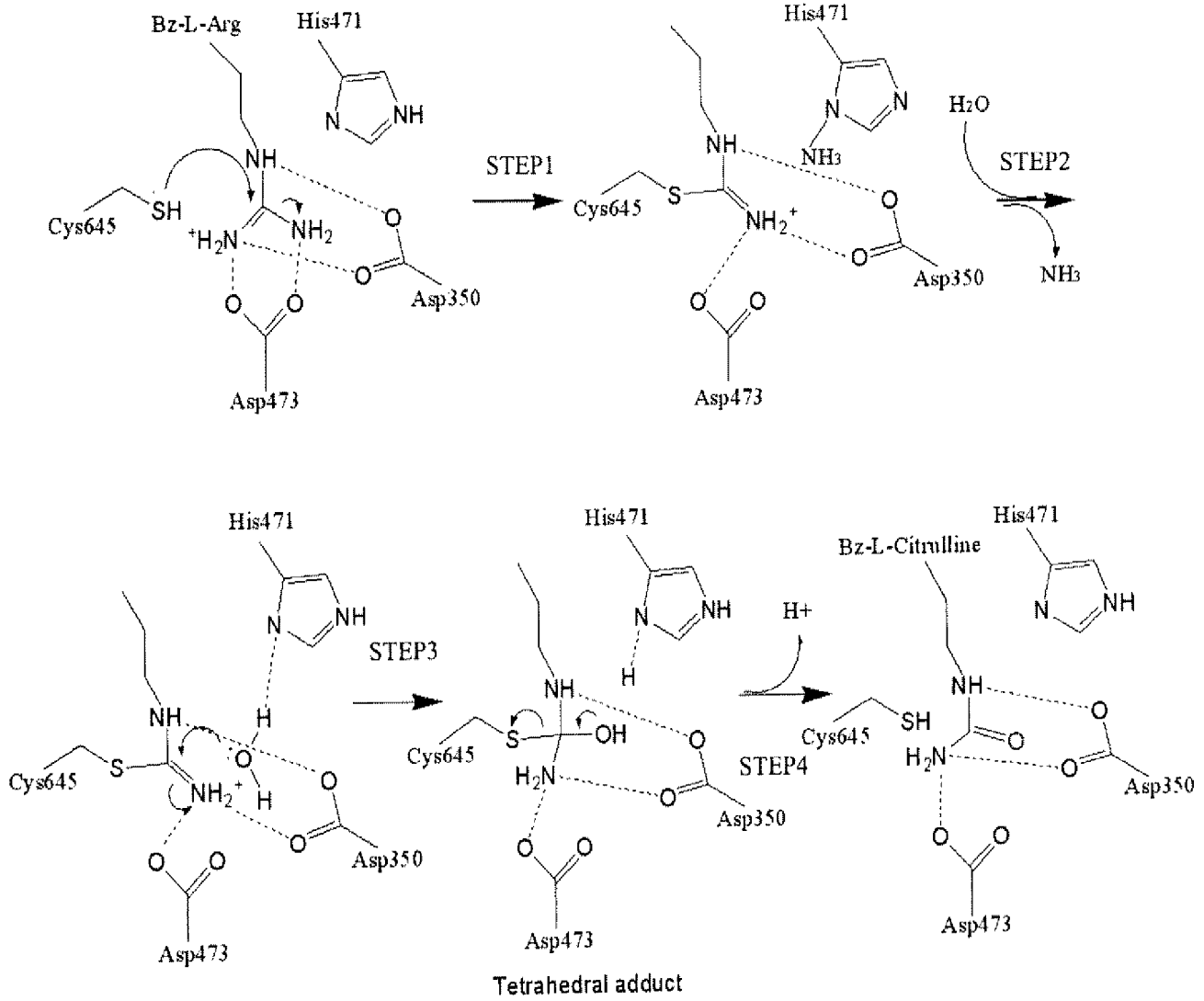
[Formula 5]



(I c)

[Claim 6] A peptidylarginine deiminase V inhibitor comprising, as the active ingredient, a substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism as shown in the following scheme between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine.

[Formula 6]


Bz-L-Arg
His471
Cys645
Asp350
Asp473
STEP1
STEP2
H2O
NH3
STEP3
STEP4
H+
Bz-L-Citrulline
Tetrahedral adduct

In the scheme, Asp350, His471, Asp473 and Cys645 represent an aspartic acid residue at position 350, a histidine residue at position 471, an aspartic acid residue at position 473 and a cysteine residue at position 645, respectively, in the amino acid sequence depicted in SEQ ID NO:1.

[Claim 7] The peptidylarginine deiminase V inhibitor according to Claim 6, wherein the substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine is an arginine derivative.

[Claim 8] A peptidylarginine deiminase V inhibitor comprising, as the active ingredient an arginine derivative having a substituent on each of the amino and guanidino groups in arginine and optionally having a substituent on the carboxyl group in arginine.

[Claim 9] The peptidylarginine deiminase V inhibitor according to Claim 7 or 8, wherein the arginine derivative is a compound or a salt thereof as recited in any one of Claims 1 to 5.

[Claim 10] The peptidylarginine deiminase V inhibitor according to any one of Claims 6 to 9, which is used for the prevention and/or treatment of a disease associated with peptidylarginine deiminase V.

[Claim 11] The peptidylarginine deiminase V inhibitor according

to Claim 10, wherein the disease associated with peptidylarginine deiminase V is rheumatoid arthritis.

[Name of Document] Specification

[Title of the Invention] PEPTIDYLARGININE DEIMINASE V INHIBITOR

Field of the Invention

[0001]

The present invention relates to peptidylarginine deiminase V inhibitors.

Background Art

[0002]

Peptidylarginine deiminase (PAD), a protein modification enzyme widely distributed throughout animal tissues, catalyzes the deimination of a peptidylarginine (protein arginine residue) to convert it into a citrulline residue in a calcium ion-dependent manner (i.e., in the presence of a calcium ion). The deimination of peptidylarginines causes a change in the distribution of positive charges in protein and, as a result, a conformational change occurs in the protein. Therefore, the deimination of a protein exerts a large influence upon the physiological functions of the protein.

[0003]

PAD was originally found in rodents, and it was demonstrated that three types of PAD were present in the tissues (non-patent documents 1, 2, 3 and 4). Afterward, Nakajima et al. detected the activity of PAD in granulocytes which had been prepared by treating human myelocytic leukemia HL-60 cells with retinoic acid, DMSO or 1,25-dihydroxyvitamin D_3 to induce the differentiation of the cells into granulocyte, and cloned the cDNA of the PAD for analysis (non-patent document 5). As a result, it was generally

revealed that the cDNA of the PAD consisted of 2238 bp and encoded 663 amino acid residues, that the amino acid sequence of the PDA was identical by about 50 to 55% to those of known types of human PAD. The PAD identified in human HL-60 cells was named "PAD V". Thereafter, PAD V was also found to be expressed in human peripheral blood granulocytes (non-patent document 6).

[0004]

To date, four types of PAD isoforms type I, II, III and IV have been identified in human (non-patent documents 7, 8, 9, 10, 11, 12, 13, 14). PAD I is involved in the differentiation of the skin (non-patent documents 15, 16 and 17), PAD II is involved in the deimination of myelin basic protein (non-patent documents 18 and 19), and PAD III is involved in the keratinization of hair follicles (non-patent documents 14, 20 and 21). PAD V, which is found in human HL-60 cells or human peripheral blood, causes the deimination of nucleophosmin B/23 and histones H2A, H3 and H4 in cells when the calcium level in the cells is increased by treating the cells with a calcium ionophore (non-patent documents 22 and 23). PAD V has a nuclear localization signal ^{56}PPAKKKST^{63}, and therefore is the only PAD isoform among the four types mentioned just above that localizes in the cell nuclei. Based on these findings, PAD V has been recognized to be a novel histone-modifying enzyme which can act on a chromatin in a calcium ion-dependent manner to regulate the nuclear functions (non-patent document 23). An amino acid sequence comparison that is made among the human PAD isoforms reveals that the isomers share high sequence homology in the C-terminal two-third region. This suggests that the PAD isoforms share the structure of the C-terminal two-third region, in which the active site of PADs is located. Recently, it has been reported that the presence of a single nucleotide

polymorphism (SNP) in the PAD V gene suppresses the mRNA decay to produce excess citrullinated proteins and thereby autoantibodies against the citrullinated proteins are formed in the blood of rheumatoid arthritis patients. This suggests that PAD V is strongly involved in the development of rheumatoid arthritis (non-patent document 24).

[0005]

Non-patent document 1: Lamensa, J. W. and Moscarello, M. A. (1993) J. Neurochem., 61, 987-996.

Non-patent document 2: Kubilus, J. and Baden, H. P. (1983) Purification and properties of a brain enzyme which deiminates proteins. Biochim. Biophys. Acta, 745, 285-291.

Non-patent document 3: Kubilus, J. and Baden, H. P. (1983) Purification and properties of a brain enzyme which deiminates proteins. Biochim. Biophys. Acta, 745, 285-291.

Non-patent document 4: Terakawa, H., Takahara, H. and Sugawara, K. (1991) Three types of mouse peptidylarginine deiminase: characterization and tissue distribution. J. Biochem. (Tokyo) 110, 661-666.

Non-patent document 5: Nakashima, K., Hagiwara, T., Ishigami, A., Nagata, S., Asaga, H., Kuramoto, M., Senshu, T. and Yamada, M. (1999) Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1α, 25-dihydroxyvitamin D3. J. Biol. Chem., 274, 27786-27792.

Non-patent document 6: Asaga, H., Nakashima, K. Senshu, T., Ishigami, A. and Yamada, M. (2001) Immunocytochemical localization of peptidylarginine deiminase in human eosinophils and neutrophils. J. Leukocyte Biol., 70, 46-51.

Non-patent document 7: Watanabe, K. and Senshu, T. (1989) J. Biol. Chem., 264, 15255-15260.

Non-patent document 8: Tsuchida, M., Takahara, H., Minami, N., Arai, T., Kobayashi, Y., Tsujimoto, H., Fukazawa, C. and Sugawara, K. (1993) Eur. J. Biochem., 215, 677-685.

Non-patent document 9: Nishijyo, T., Kawada, A., Kanno, T., Shiraiwa, M. and Takahara, H. (1997) J. Biochem. (Tokyo) 121, 868-875.

Non-patent document 10: Yamakoshi, A., Ono, H., Nishijyo, T., Shiraiwa, M. and Takahara, H. (1998) Biochim. Biophys. Acta, 1386, 227-232.

Non-patent document 11: Ishigami, A., Kuramoto, M., Yamada, M., Watanabe, K. and Senshu, T. (1998) FEBS Lett., 433, 113-118.

Non-patent document 12: Rus'd, A. A., Ikejiri, Y., Ono, H., Yonekawa, T., Shiraiwa, M., Kawada, A. and Takahara, H. (1999) Eur. J. Biochem., 259, 660-669.

Non-patent document 13: Nakashima, K., Hagiwara, T., Ishigami, A., Nagata, S., Asaga, H., Kuramoto, M., Senshu, T. and Yamada, M. (1999) Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1α, 25-dihydroxyvitamin D3. J. Biol. Chem., 274, 27786-27792.

Non-patent document 14: Kanno, T., Kawada, A., Yamanouchi, J., Yosida-Noro, C., Yoshiki, A., Siraiwa, M., Kusakabe, M., Manabe, M., Tezuka, T. and Takahara, H. (2000) J. Invest. Dermatol., 115, 813-823.

Non-patent document 15: Senshu, T., Akiyama, K., Kan, S., Asaga, H., Ishigami, A. and Manabe, M. (1995) J. Invest. Dermatol., 105, 163-169.

Non-patent document 16: Senshu, T., Akiyama, K., Ishigami, A. and Nomura, K. (1999) J. Dermatol. Sci., 21, 113-126.

Non-patent document 17: Ishida-Yamamoto, A., Senshu, T., Eady, R. A., Takahashi, H., Shimizu, H., Akiyama, M. and Iizuka,

H. (2002) J. Invest. Dermatol., 118, 282-287.

Non-patent document 18: Pritzker LB, Nguyen TA, Moscarello MA. (1997) The developmental expression and activity of peptidylarginine deiminase in the mouse. Neurosci Lett.266, 161-164.

Non-patent document 19: Moscarello MA, Pritzker L, Mastronardi FG, Wood DD. Peptidylarginine deiminase: a candidate factor in demyelinating disease. J Neurochem. 81, 335-43.

Non-patent document 20: Rogers, G., Winter, B., McLaughlan, C., Powell, B. and Nesci, T. (1997) J. Invest. Dermatol., 108, 700-707.

Non-patent document 21: Ohsawa, T., Ishigami, A., Akiyama, K. and Asaga, H. (2001) Biomed. Res., 22, 91-97, Pritzker, L. B., Nguyen, T. A. and Moscarello, M. A. (1999) Neurosci. Lett., 266, 161-164.

Non-patent document 22: Hagiwara, T., Nakashima, K., Hirano, H., Senshu, T. and Yamada, M. (2002) Biochem. Biophys. Res. Commun. 290, 979-983.

Non-patent document 23: Nakashima K, Hagiwara T, Yamada M. (2002) Nuclear localization of peptidylarginine deiminase V and histone deimination in granulocytes. J. Biol. Chem., 277, 49562-49568.

Non-patent document 24: Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y. and Yamamoto, K. (2003) Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase V, are associated with rheumatoid

arthritis. Nature Genetics, 34, 395-402.

Disclosure of the Invention

Problems to be Solved by the Invention

[0006]

The object of the present invention is to design a novel substance capable of inhibiting the enzymatic activity of PAD V and to develop a new drug against rheumatoid arthritis.

Means for Solving the Problems

[0007]

The present inventors used X-ray diffraction at resolutions of 2.80 and 2.5 angstroms, respectively to determine the three-dimensional structures of human PAD V in the absence of calcium ions, and calcium ions and substrate (benzoyl-L-arginine: BA)-bound mutant PAD V whose enzymatic activity was completely inactivated by substitution of Ala for Cys645 (Japanese Patent Application No. 2003-358459). The conformations of the two proteins were almost the same except for the region surrounding the active site including the calcium-bound sites. A PAD V molecule had an elongated boot-like shape, and was related with the most proximal molecule in the crystal lattice by a crystallographic two-fold axis to form a functional dimer. The PAD V molecule was dividable into two domains, the N-terminal domain and the C-terminal domain. The N-terminal domain was further divided into two sub-domains which, when combined; resembled in structure the T-cell surface glycoprotein CD4 that had an immunoglobulin-like structure, with one sub-domain also resembling in structure the DNA-binding domain of p53. The C-terminal domain, on the other hand, was composed of five

ββαβ-propeller structures and had a negatively charged large groove at the center. The groove included four active residues Asp350, His471, Asp473 and Cys645, and calcium ions, with the topology around the active residues being similar to those of amidinotransferase (AT) and N(G),N(G)-dimethyl-L-arginine aminidinohydrorase. The calcium ions are bound to Asn349, Glu353, Phe407, Lue410, and Glu411. The structure around the active residues was compared with that of Ca^{2+}-free PAD V, revealing that binding of the calcium ions caused a significant change in the structure around C645 (A645) and Asp350. It was also found that the manner of binding of each calcium ion was distinctly different from that of a well-known EF-hand motif. From these findings, it was demonstrated that PAD V, although being a protein in a superfamily of arginine-modifying enzymes, is an entirely new calcium-dependent protein modification enzyme whose catalytic activity is controlled by calcium ions and whose binding mode is different from that of a protein having an EF-hand motif known as a calcium-binding motif. Additionally, the comparison between the structure of Ca^{2+}-free PAD V and that of BA-Ca^{2+} PAD V (C645A) revealed that the structures are changed by binding of the calcium ions so that the substrate molecule can be bound at an active site, indicating that the calcium ions recognize a substrate binding and control PAD V activity.

[0008]

Using programs PSI-BLAST and FUGUE, Shirai et al. speculated that arginine modifying enzymes would share a common fold and proposed a reaction mechanism for deimination of arginine (Shirai, H., Blundell, T. L. and Mizuguchi, K. (2001) A novel superfamily of enzymes that catalyze the modification of guanidino groups. TIBS, 26, 465-468). The present inventors made a structural

analysis of BA-Ca^{2+} PAD V (C645A), demonstrating that the deimination reaction mechanism of arginine was consistent with that proposed by Shirai et al. Therefore, it is assumed that the deimination of protein by PAD V occurs through the two-stage reaction mechanism proposed by Shirai et al. That is, in the first stage, the carbon Cζ in the guanidino group is added to a thiol group of Cys645, whereby a proton is donated to arginine. Next, the nitrogen atom in the guanidino group forms hydrogen bonds with Asp350 and Asp473, whereby the electrophilicity of the guanidino group is increased and the hydrogen bond between Nη1 and His471 aids the transfer of this proton to cleave the bond of the aminido carbon Cζ and Nη. In the second stage, a proton transfers from a water molecule to His471 and subsequently a lone pair in the oxygen atom in the water molecule nucleophilically attacks the amidino carbon Cζ. As a result, it is believed that a tetrahedral polymer is formed and the binding between the amidino carbon Cζ and the sulfur atom Sγ in Cys645 is cleaved to produce a citrulline residue. The PAD V deimination mechanism proposed by the present inventors is shown in Fig. 1.

[0009]

Based on the findings mentioned above, the present inventors designed and synthesized novel compounds capable of inhibiting the enzymatic activity of PAD V and measured the PAD V-inhibition activities of the compounds. As a result, it was found that the compounds possessed a PAD V-inhibition activity, which has led to the accomplishment of the present invention.

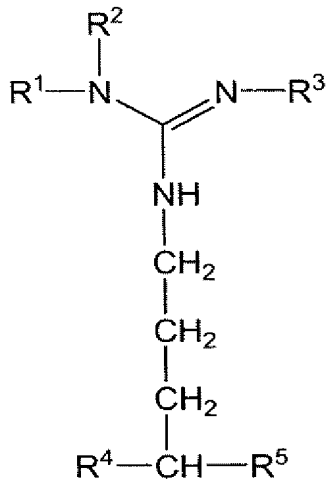
[0010]

The aspects of the present invention are as follows.

(1) A compound represented by the general formula (I) or a salt thereof:

[0011]

[Formula 7]



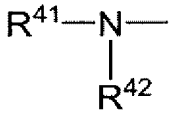
(I)

wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or an alkyl group having 1 to 3 carbon atoms, provided that at least one of R^1, R^2 and R^3 does not represent a hydrogen atom; R^4 represents an amino group which has a substituent; and R^5 represents a carboxyl group which may have a substituent.

(2) The compound or salt thereof according to item (1), wherein R^4 represents the following formula:

[0012]

[Formula 8]



wherein R^{41} represents a group represented by $R^{401}CO-$ where R^{401} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent,

a group represented by $R^{402}S(O)_m$- where R^{402} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and m is an integer of 1 or 2, or a group represented by $R^{405}N(R^{406})$-CHR^{404}-CO-$[NH-CHR^{403}-CO]_n$- where R^{403}, R^{404}, R^{405} and R^{406} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and n is an integer of 1 to 50; and R^{42} represents a hydrogen atom or an alkyl group having 1 to 3 carbon atoms.

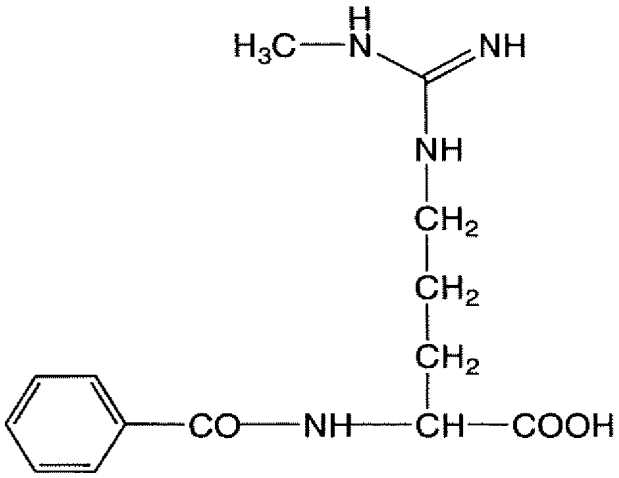
(3) The compound or salt thereof according to item (2), wherein R^{41} represents a benzoyl group which may have a substituent, a benzoylpeptidyl group which may have a substituent, a dansyl group which may have a substituent or a dansylpeptidyl group which may have a substituent; and R^{42} represents a hydrogen atom.

(4) The compound or salt thereof according to any one of items (1) to (3), wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or a methyl group, provided that at least one of R^1, R^2 and R^3 represents a methyl group.

(5) The compound or salt thereof according to Claim 4, which is a compound represented by the formula (Ia), (Ib) or (Ic) or a salt thereof.

[0013]

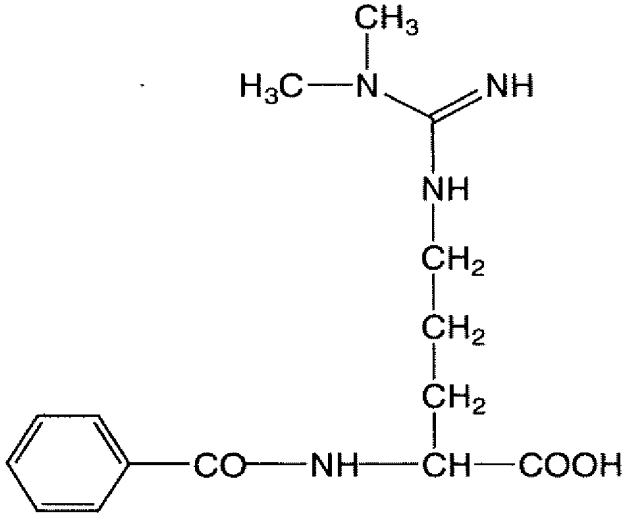
[Formula 9]



(Ia)

[0014]

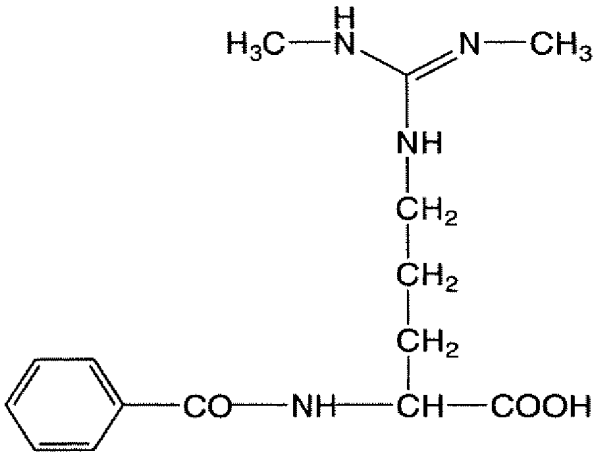
[Formula 10]



(Ib)

[0015]

[Formula 11]

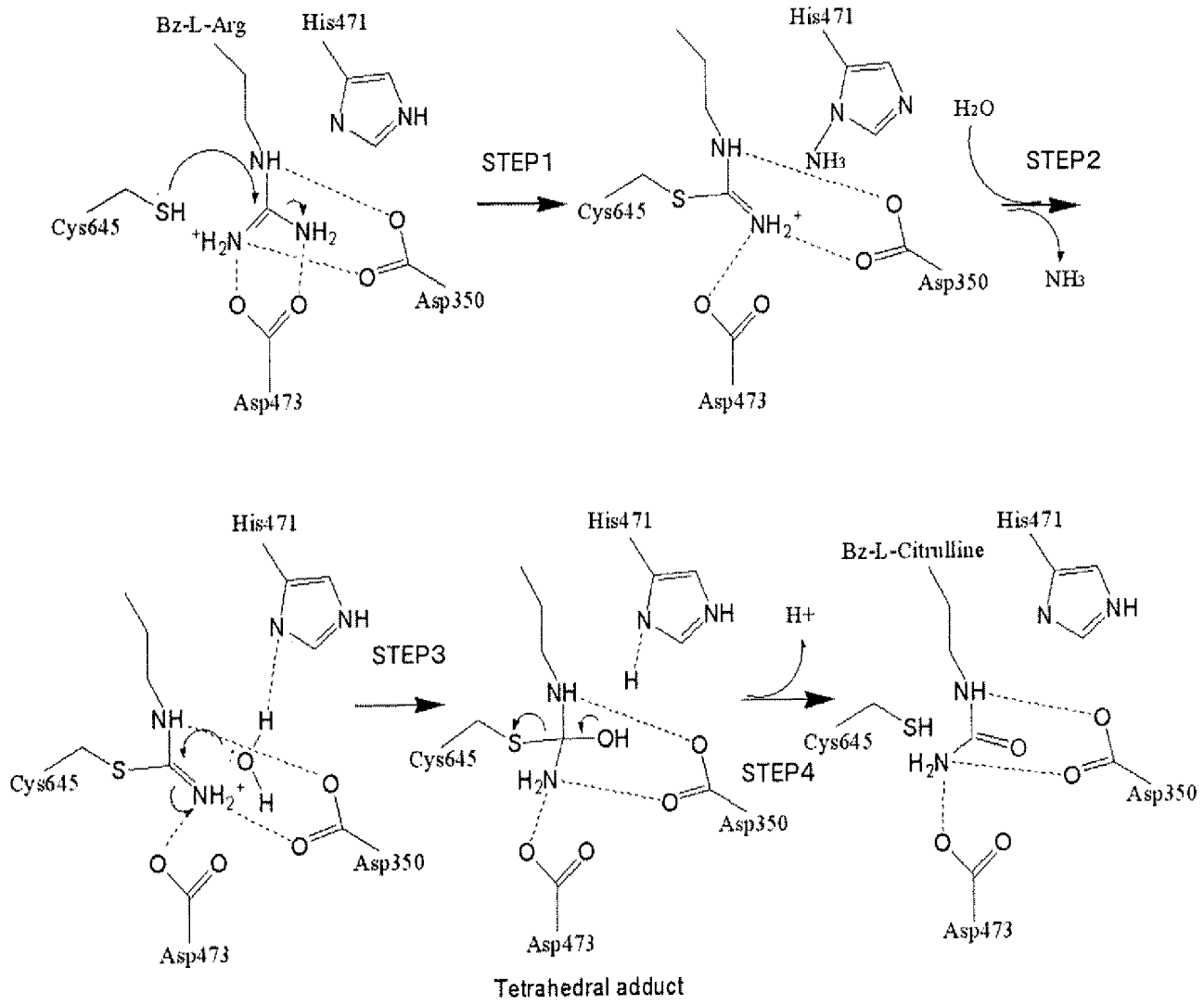


(Ic)

(6) A peptidylarginine deiminase V inhibitor comprising as the active ingredient a substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism as shown in the following scheme between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine.

[0016]

[Formula 12]


Bz-L-Arg
His471
Cys645
Asp350
Asp473
STEP1
STEP2
H2O
NH3
STEP3
STEP4
H+
Bz-L-Citrulline
Tetrahedral adduct

In the scheme, Asp350, His471, Asp473 and Cys645 represent an aspartic acid residue at position 350, a histidine residue at position 471, an aspartic acid residue at position 473 and a cysteine residue at position 645, respectively, in the amino acid sequence depicted in SEQ ID NO:1.

(7) The peptidylarginine deiminase V inhibitor according to item (6), wherein the substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine is an arginine derivative.

(8) A peptidylarginine deiminase V inhibitor comprising as the active ingredient an arginine derivative having a substituent on each of the amino and guanidino groups in arginine and optionally having a substituent on the carboxyl group in arginine.

(9) The peptidylarginine deiminase V inhibitor according to item (7) or (8), wherein the arginine derivative is a compound or a salt thereof as recited in any one of items (1) to (5).

(10) The peptidylarginine deiminase V inhibitor according to any one of items (6) to (9), which is used for the prevention and/or treatment of diseases associated with peptidylarginine deiminase V.

(11) The peptidylarginine deiminase V inhibitor according to item (10), wherein the diseases associated with peptidylarginine deiminase V is rheumatoid arthritis.

[0017]

As used herein, the term "peptidylarginine deiminase V" refers to wild type peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1, and includes analogous

substances having a similar biological activity (i.e., the enzymatic activity of catalyzing the reaction for deiminating an arginine residue in a protein into a citrulline residue in the presence of a calcium ion) and which also have amino acid sequences homologous to the amino acid sequence depicted in SEQ ID NO:1.

[0018]

As used herein, "Boc" represents a t-butoxy group, "Arg" represents arginine, "Tos" represents p-toluenesulfonyl, "Me" represents a methyl group, "ADMA" represents N^G,N^G-dimethyl-L-arginine, "SDMA" represents $N^G,N^{G'}$-dimethyl-L-arginine", and "Bz" represents a benzoyl group.

[0019]

As used herein, the symbol "-" means a specified range including the numerical values both before and after the symbol as the minimal and maximum values, respectively.

[0020]

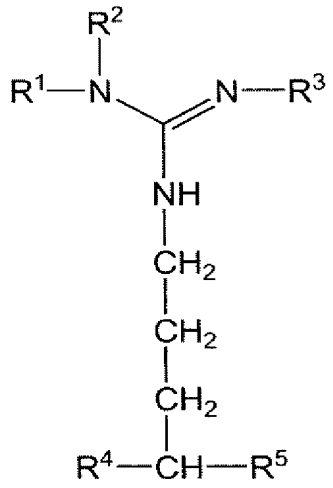
Hereinbelow, the present invention will be described in detail.

1. Compounds represented by the general formula (I) or salt thereof

The present invention provides a compound represented by the general formula (I) or a salt thereof.

[0021]

[Formula 13]



(I)

The compound of the general formula (I) or the salt thereof may be of L-, D- or DL-form, but an L-form is effective.

[0022]

In the general formula (I), R^1, R^2 and R^3 each independently represent a hydrogen atom or an alkyl group having 1 to 3 carbon atoms, provided that at least one of R^1, R^2 and R^3 is not a hydrogen atom. Examples of the alkyl group having 1 to 3 carbon atoms include methyl, ethyl, n-propyl and i-propyl groups.

[0023]

Preferably, R^1, R^2 and R^3 each independently represent a hydrogen atom or a methyl group, provided that at least one of R^1, R^2 and R^3 is a methyl group.

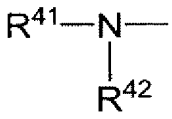
[0024]

In the general formula (I), R^4 represents an amino group which has a substituent. The substituent to be added to the amino group for R^4 may be of any type, as long as a compound having the substituent can be recognized by PAD V (i.e., the compound can interact with PAD V). Preferably, the substituent is one having

an oxo group (=O) attached to the atom which is directly bound to the nitrogen in the amino group for R^4. One example of R^4 is a group represented by the following formula.

[0025]

[Formula 14]



[0026]

In the formula above, R^{41} represents a group represented by $R^{401}CO-$ where R^{401} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, a group represented by $R^{402}S(O)_m-$ where R^{402} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and m is an integer of 1 or 2, or a group represented by $R^{405}N(R^{406})-CHR^{404}-CO-[NH-CHR^{403}-CO]_n-$ where R^{403}, R^{404}, R^{405} and R^{406} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and n is an integer of 1 to 50; and R^{42} represents a hydrogen atom or an alkyl group having 1 to 3 carbon atoms. Examples of the group represented by $R^{405}N(R^{406})-CHR^{404}-CO-$ and the group represented by $-NH-CHR^{403}-CO-$ include amino acid residues occurring in natural proteins and peptides.

[0027]

Examples of the hydrocarbon group for R^{401}, R^{402}, R^{403}, R^{404}, R^{405} and R^{406} include a saturated chain hydrocarbon group (e.g., a straight-chain or branched alkyl group having 1 to 6 carbon

atoms), an unsaturated chain hydrocarbon group (e.g., a straight-chain or branched alkenyl group having 1 to 6 carbon atoms, a straight-chain or branched alkynyl group having 1 to 6 carbon atoms), an alicyclic hydrocarbon group (e.g., a cycloalkyl group having 1 to 6 carbon atoms, a cycloalkenyl group having 1 to 6 carbon atoms, a cycloalkynyl group having 1 to 6 carbon atoms) and an aromatic hydrocarbon group (e.g., phenyl, naphthyl, anthryl and phenanthryl groups).

[0028]

When R^{401}, R^{402}, R^{403}, R^{404}, R^{405} or R^{406} is a hydrocarbon group which may have a substituent, examples of the substituent include a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a hydroxyl group, an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, pentoxy), an amino group, a carbamoyl group, an alkoxycarbonyl group having 1 to 6 carbon atoms (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), and a heterocyclic group (examples of the heterocyclic ring in the heterocyclic group include a 5- to 7-membered ring having one sulfur, nitrogen or oxygen atom, a 5- to 6-membered ring having 2 to 4 nitrogen atoms, and a 5- to 6-membered ring having one or two nitrogen atoms and one sulfur or oxygen atom, these heterocyclic rings being optionally fused to a 6-membered ring having one or two nitrogen atoms, a benzene ring or a 5-membered ring having one sulfur atom; specific examples of the heterocyclic group include 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrido[2,3-d]pyrimidyl, benzopyranyl, 1,8-naphthyridyl, 1,5-naphthyridyl, 1,6-naphthyridyl, 1,7-naphthyridyl, quinolyl, thieno[2,3-b]pyridyl, tetrazolyl, thiadiazolyl, oxadiazolyl,

triazinyl, triazolyl, thienyl, pyrrolyl, pyrrolinyl, furyl, pyrrolidinyl, benzothienyl, indolyl, imidazolidinyl, piperidyl, piperidino, piperazinyl, morpholinyl and morpholino). The amino group may be substituted by an alkyl group having 1 to 6 carbon atoms or an acyl group having 1 to 10 carbon atoms. The carbamoyl group may be substituted by an alkyl group having 1 to 6 carbon atoms.

[0029]

Examples of the heterocyclic ring in the heterocyclic group for R^{401}, R^{402}, R^{403}, R^{404}, R^{405} or R^{406} include a 5- to 7-membered ring having one sulfur, nitrogen or oxygen atom, a 5- to 6-membered ring having 2 to 4 nitrogen atoms, and a 5- to 6-membered ring having one or two nitrogen atoms and one sulfur or oxygen atom, these heterocyclic rings being optionally fused to a 6-membered ring having one or two nitrogen atoms, a benzene ring or a 5-membered ring having one sulfur atom. Specific examples of the heterocyclic group include 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrido[2,3-d]pyrimidyl, benzopyranyl, 1,8-naphthyridyl, 1,5-naphthyridyl, 1,6-naphthyridyl, 1,7-naphthyridyl, quinolyl, thieno[2,3-b]pyridyl, tetrazolyl, thiadiazolyl, oxadiazolyl, triazinyl, triazolyl, thienyl, pyrrolyl, pyrrolinyl, furyl, pyrrolidinyl, benzothienyl, indolyl, imidazolidinyl, piperidyl, piperidino, piperazinyl, morpholinyl and morpholino.

[0030]

When R^{401}, R^{402}, R^{403}, R^{404}, R^{405} or R^{406} is a heterocyclic group which may have a substituent, examples of the substituent include a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a hydroxyl group, an alkyl group having 1 to 6 carbon atoms (e.g.,

methyl, ethyl, n-propyl, i-propyl), an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, pentoxy), an amino group, a carbamoyl group, an alkoxycarbonyl group having 1 to 6 carbon atoms (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), and a heterocyclic ring as mentioned above. The amino group may be substituted by an alkyl group having 1 to 6 carbon atoms or an acyl group having 1 to 10 carbon atoms. The carbamoyl group may be substituted by an alkyl group having 1 to 6 carbon atoms.

[0031]

Examples of the alkyl group having 1 to 3 carbon atoms for R^{42} include methyl, ethyl, n-propyl and i-propyl groups.

[0032]

Preferably, R^{41} is a benzoyl group which may have a substituent, a benzoylpeptidyl group which may have a substituent, a dansyl group which may have a substituent or a dansylpeptidyl group which may have a substituent, and R^{42} is a hydrogen atom.

[0033]

In the general formula (I), R^5 is a carboxyl group which may have a substituent. When R^5 is a carboxyl group which has a substituent, the substituent may be of any type. For example, in order to increase the inhibitory activity against PAD V, R^5 is preferably a group represented by $-COOR^{51}$ wherein R^{51} represents an alkyl group having 1 to 20 carbon atoms, a group represented by $-COO-\{R^{54}N(R^{55})-CHR^{53}-CO-[NH-CHR^{52}-CO]_p-\}$ wherein R^{52}, R^{53}, R^{54} and R^{55} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and p is an integer of 1 to 50, or like groups. Examples of the group represented by $R^{54}N(R^{55})-CHR^{53}-CO-$ and the group represented by $-NH-CHR^{52}-CO-$ include amino acid

residues occurring in natural proteins and peptides.

[0034]

The alkyl group for R^{51} may be either straight-chain or branched alkyl group having 1 to 20 carbon atoms, and may specifically be exemplified by methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, tert-butyl, pentyl, isopentyl, neopentyl, hexyl, heptyl, octyl, nonyl and decyl groups.

[0035]

Examples of the hydrocarbon group for R^{52}, R^{53}, R^{54} and R^{55} include a saturated chain hydrocarbon group (e.g., a straight-chain or branched alkyl group having 1 to 6 carbon atoms), an unsaturated chain hydrocarbon group (e.g., a straight-chain or branched alkenyl group having 1 to 6 carbon atoms, a straight-chain or branched alkynyl group having 1 to 6 carbon atoms), an alicyclic hydrocarbon group (e.g., a cycloalkyl group having 1 to 6 carbon atoms, a cycloalkenyl group having 1 to 6 carbon atoms, a cycloalkynyl group having 1 to 6 carbon atoms) and an aromatic hydrocarbon group (e.g., phenyl, naphthyl, anthryl and phenanthryl groups).

[0036]

When R^{52}, R^{53}, R^{54} or R^{55} is a hydrocarbon group which may have a substituent, examples of the substituent include a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a hydroxyl group, an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, pentoxy), an amino group, a carbamoyl group, an alkoxycarbonyl group having 1 to 6 carbon atoms (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), and a heterocyclic group (examples of the heterocyclic ring in the heterocyclic group include a 5- to 7-membered ring having one sulfur, nitrogen or oxygen atom, a 5- to 6-membered ring having

2 to 4 nitrogen atoms, and a 5- to 6-membered ring having one or two nitrogen atoms and one sulfur or oxygen atom, these heterocyclic rings being optionally fused to a 6-membered ring having one or two nitrogen atoms, a benzene ring or a 5-membered ring having one sulfur atom; specific examples of the heterocyclic group include 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrido[2,3-d]pyrimidyl, benzopyranyl, 1,8-naphthyridyl, 1,5-naphthyridyl, 1,6-naphthyridyl, 1,7-naphthyridyl, quinolyl, thieno[2,3-b]pyridyl, tetrazolyl, thiadiazolyl, oxadiazolyl, triazinyl, triazolyl, thienyl, pyrrolyl, pyrrolinyl, furyl, pyrrolidinyl, benzothienyl, indolyl, imidazolidinyl, piperidyl, piperidino, piperazinyl, morpholinyl and morpholino). The amino group may be substituted by an alkyl group having 1 to 6 carbon atoms or an acyl group having 1 to 10 carbon atoms. The carbamoyl group may be substituted by an alkyl group having 1 to 6 carbon atoms.

[0037]

The heterocyclic ring in the heterocyclic group for R^{52}, R^{53}, R^{54} or R^{55} may be exemplified by a 5- to 7-membered ring having one sulfur, nitrogen or oxygen atom, a 5- to 6-membered ring having 2 to 4 nitrogen atoms, and a 5- to 6-membered ring having one or two nitrogen atoms and one sulfur or oxygen atom, these heterocyclic rings being optionally fused to a 6-membered ring having one or two nitrogen atoms, a benzene ring or a 5-membered ring having one sulfur atom. Specific examples of the heterocyclic group include 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, pyrazolyl, imidazolyl, thiazolyl, isothiazolyl, oxazolyl, isoxazolyl,

pyrido[2,3-d]pyrimidyl, benzopyranyl, 1,8-naphthyridyl, 1,5-naphthyridyl, 1,6-naphthyridyl, 1,7-naphthyridyl, quinolyl, thieno[2,3-b]pyridyl, tetrazolyl, thiadiazolyl, oxadiazolyl, triazinyl, triazolyl, thienyl, pyrrolyl, pyrrolinyl, furyl, pyrrolidinyl, benzothienyl, indolyl, imidazolidinyl, piperidyl, piperidino, piperazinyl, morpholinyl and morpholino.

[0038]

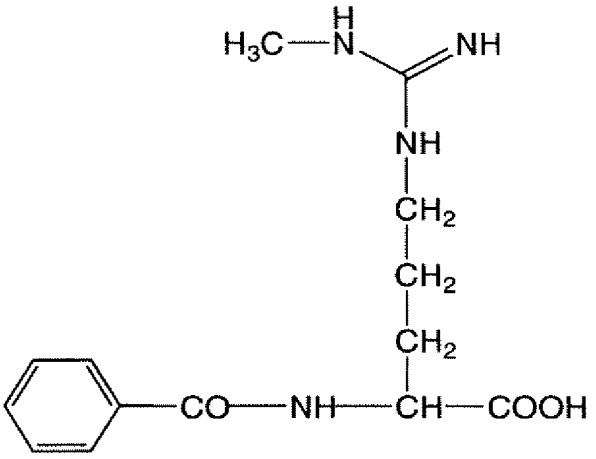
When R^{52}, R^{53}, R^{54} or R^{55} is a heterocyclic group which may have a substituent, examples of the substituent include a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a hydroxyl group, an alkyl group having 1 to 6 carbon atoms (e.g., methyl, ethyl, n-propyl and i-propyl), an alkoxy group having 1 to 6 carbon atoms (e.g., methoxy, ethoxy, propoxy, butoxy, pentoxy), an amino group, a carbamoyl group, an alkoxycarbonyl group having 1 to 6 carbon atoms (e.g., methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl), and a heterocyclic group as mentioned above. The amino group may be substituted by an alkyl group having 1 to 6 carbon atoms or an acyl group having 1 to 10 carbon atoms. The carbamoyl group may be substituted by an alkyl group having 1 to 6 carbon atoms.

[0039]

Specific examples of the compound represented by the general formula (I) include compounds represented by the following formulae (Ia), (Ib) and (Ic).

[0040]

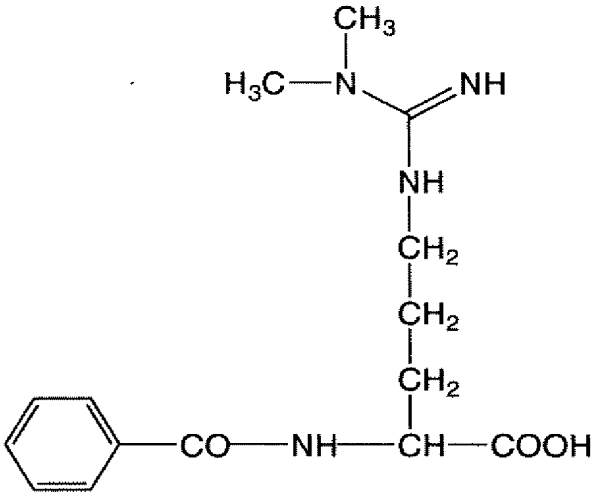
[Formula 15]



(Ia)

[0041]

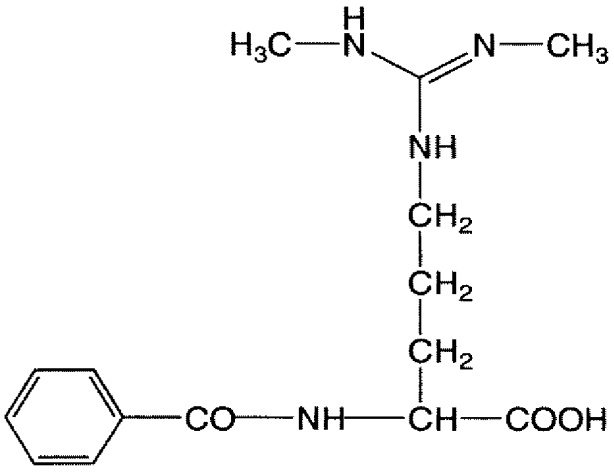
[Formula 16]



(Ib)

[0042]

[Formula 17]



(Ic)

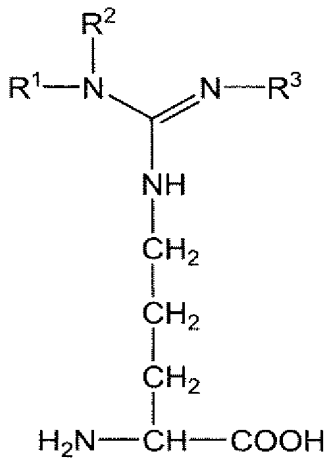
The compound represented by the formula (Ia) is Bz-Arg (mono-methyl). The compound represented by the formula (Ib) is Bz-ADMA. The compound represented by the formula (Ic) is Bz-SDMA.

[0043]

The compound represented by the general formula (I) can be synthesized starting from commercially available arginine or an arginine derivative represented by the following formula.

[0044]

[Formula 18]



wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or an alkyl group having 1 to 3 carbon atoms, provided that at least one of R^1, R^2 and R^3 is not a hydrogen atom.

[0045]

A compound of the general formula (I) wherein R^4 is a group represented by R^{401}-CO-NH- where R^{401} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and wherein R^5 represents a carboxyl group, can be produced by acylation of the starting material (i.e., arginine or the arginine derivative mentioned above) with a symmetric acid anhydride represented by $R^{401}CO\text{-}O\text{-}COR^{401}$ or by benzoylation of the starting material with Bz_2O (benzoic anhydride). The benzoylation reaction can be performed in any known manner. For example, the benzoylation reaction may be performed in an inert solvent in the presence of a base. The inert solvent to be used in this reaction may be exemplified by dimethylformamide (DMF), dimethyl sulfoxide

(DMSO) and tetrahydrofuran (THF), which may be mixed with water or with themselves. As for the base, sodium hydrogencarbonate or potassium hydrogencarbonate may be used so that the pH of the reaction solution is adjusted to about 10 or lower in view of the fact that the pKa value of the guanidino skeleton in the arginine side chain is about 12. The reaction temperature is preferably about 0 to 37°C, and the reaction time is preferably about 10 minutes to about 24 hours. The amount of Bz_2O to be used is preferably about 1 to 1.2 moles per mole of arginine or the arginine derivative (starting material) to be used.

[0046]

Speaking of a compound of the general formula (I) wherein R^4 is a group represented by R^{402}-$S(O)_m$-NH- where R^{402} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and m is an integer of 1 or 2, and wherein R^5 represents a carboxyl group, it can, if m = 2, be produced by dansylation of the starting materinal (i.e., arginine or the arginine derivative mentioned above) with DNS-Cl (dansyl chloride). The dansylation reaction can be performed in any known manner (B.S. Hartley, V. Massey, Biochim. Biophys. Acta, 21, 58 (1956)). For example, the dansylation reaction may be performed in an inert solvent in the presence of a base. The inert solvent to be used in this reaction may be exemplified by acetone, dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF), which may be mixed with water or with themselves. As for the base, sodium hydrogencarbonate or potassium hydrogencarbonate may be used so that the pH of the reaction solution is adjusted to about 10 or lower in view of the fact that the pKa value of the guanidino skeleton in the arginine side chain is about 12. The reaction

temperature is preferably about 0 to 37°C, and the reaction time required is preferably about 10 minutes to about 24 hours. The amount of DNS-Cl to be used is preferably about 1 to 1.2 moles per mole of arginine or the arginine derivative (starting material) and its concentration is desirably about 5 mM.

[0047]

A compound of the general formula (I) wherein R^4 is a group represented by $R^{405}N(R^{406})$-CHR^{404}-CO-[NH-CHR^{403}-CO]$_n$-NH- where R^{403}, R^{404}, R^{405} and R^{406} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent and n is an integer of 1 or 50, and wherein R^5 represents a carboxyl group, can be produced by the following exemplary method. First, arginine or the arginine derivative described above (starting material) is butyloxycarbonylated with Boc_2O (t- butyloxycarbonylated symmetric acid anhydride) in the same manner as in the benzoylation mentioned above. Boc-Arg or a derivative thereof produced by this reaction is then treated with p-toluenesulfonyl chloride to tosylate the guanidino group in the side chain in accordance with a known method (J. Ramachandran, C.H. Li, J. Org. Chem., 27, 4006 (1962)). The peptide can be produced by using this derivative according to a known method, or the so-called solid-phase synthesis method for peptide (awarded the Nobel Prize in Chemistry) (R.B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963)).

[0048]

Speaking of a compound of the general formula (I) wherein R^4 is a group represented by R^{401}-CO-NR^{42} where R^{401} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and wherein R^5 represents a carboxyl group, it can, if R^{42} is a methyl

group(-CH_3), be synthesized by treating an N^{α}-methyl form of arginine or the arginine derivative (as a starting material) with a symmetric acid anhydride represented by $R^{401}CO\text{-}O\text{-}COR^{401}$. For example, the reaction may be performed in an inert solvent in the presence of a base. The inert solvent to be used in this reaction may be exemplified by dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF), which may be mixed with water or with themselves. As for the base, sodium hydrogencarbonate or potassium hydrogencarbonate may be used so that the pH of the reaction solution is adjusted to about 10 or lower in view of the fact that the pKa value of the guanidino skeleton in the arginine side chain is about 12. The reaction temperature is preferably about 0 to 37°C, and the reaction time is preferably about 10 minutes to about 24 hours. The amount of the symmetric acid anhydride to be used is preferably about 1 to 1.2 moles per mole of the N^{α}-methyl form of arginine or the arginine derivative (starting material).

[0049]

As the starting material for the synthesis of a compound of the general formula (I) wherein R^{42} is a methyl group (CH_3-), Boc-N-Me-Arg(Tos)-OH is commercially available from BACHEM AG. This compound is treated with trifluoroacetic acid to remove the Boc group, thereby producing N-Me-Arg(Tos)-OH (Text for Biochemical Experiments Vol.1, Chemistry of Proteins IV -Chemical Modification and Peptide Synthesis-, p.234, ed. the Society of Biochemistry, Japan, published by Tokyo Kagaku-Dojin, Tokyo, Japan). This product may be treated with a symmetric acid anhydride or Bz_2O to modify the α-amino group in the methyl form in various manners.

[0050]

A compound of the general formula (I) wherein the guanidino group in the side chain is methylated and the α-amino group is methylated can be synthesized as follows. First, commercially available Arg (mono-methyl), ADMA or SDMA is butyloxycarbonylated (T. Nagasawa, K. Kuroiwa, K. Narita, Y. Isowa, Bull. Chem. Soc. Jpn., 46, 1269 (1973)) to produce Boc-Arg (mono-methyl), Boc-ADMA or Boc-SDMA. Next, the methylated guanidino group in the side chain is further tosylated (J. Ramachandran, C. H. Li, J. Org. Chem., 27, 4006 (1962)) to prepare the respective tosylated form, Boc-Arg(mono-methyl,Tos), Boc-ADMA(Tos) or Boc-SDMA(Tos). This compound is treated with trifluoroacetic acid to remove the Boc group to produce Arg(mono-methyl,Tos), ADMA(Tos) or SDMA(Tos). The resulting product is used as a starting material and converted into an N-benzylideneamino acid, which is then reduced into an N-benzylated compound. The N-benzylated compound is methylated with formalin and formic acid and then subjected to catalytic reduction to remove the benzyl group, thereby producing N-Me-Arg(mono-methyl,Tos), N-Me-ADMA(Tos) or N-Me-SDMA(Tos) (P. Quitt, J. Hellerbach, K. Volger, Helv. Chim. Acta, 46, 327 (1963)). This product is treated with HF as described above (S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, H. Sugihara, Bull. Chem. Soc. Jpn, 40, 2164 (1967)) to produce N-Me-Arg (mono-methyl), N-Me-ADMA or N-Me-SDMA. The compound may be used as a starting material which is treated with a symmetric acid anhydride or Bz_2O to modify the α-amino group in the methyl form in various manners.

[0051]

Speaking of a compound of the general formula (I) wherein R^4 is a group represented by R^{402}-$S(O)_m$-NR^{42}- where R^{402} represents a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent, and m is

an integer of 1 or 2, and wherein R^5 represents a carboxyl group, it can, if m = 2 and R^{42} is a methyl group (CH_3-), be produced by dansylation of an N^{α}-methyl form of a starting substance (i.e., arginine or the arginine derivative mentioned above) with DNS-Cl (dansyl chloride). The dansylation reaction can be performed in any known manner (B.S. Hartley, V. Massey, Biochim. Biophys. Acta, 21, 58 (1956)). For example, the dansylation reaction may be performed in an inert solvent in the presence of a base. The inert solvent to be used in this reaction may be exemplified by acetone, dimethylformamide (DMF), dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF), which may be mixed with water or with themselves. As for the base, sodium hydrogencarbonate or potassium hydrogencarbonate may be used so that the pH of the reaction solution is adjusted to about 10 or lower in view of the fact that the pKa value of the guanidino skeleton in the arginine side chain is about 12. The reaction temperature is preferably about 0 to 37°C, and the reaction time is preferably about 10 minutes to about 24 hours. The amount of DNS-Cl to be used is preferably about 1 to 1.2 moles per mole of the N^{α}-methyl form of arginine or the arginine derivative (starting material) and its concentration is desirably about 5 mM.

[0052]

Speaking of a compound of the general formula (I) wherein R^4 is a group represented by $R^{405}N(R^{406})$-CHR^{404}-CO-$[NH-CHR^{403}-CO]_n$-NR^{42}- where R^{403}, R^{404}, R^{405} and R^{406} each independently represent a hydrogen atom, a hydrocarbon group which may have a substituent or a heterocyclic group which may have a substituent and n is an integer of 1 or 50, and wherein R^5 represents a carboxyl group, it can, if R^{42} is a methyl group (CH_3-), be produced by butyloxylcarbonylation of an N^{α}-methyl form

of a starting material (i.e., arginine or the arginine derivative mentioned above) with Boc_2O (a t-butyloxycarbonylated symmetric acid anhydride) in the same manner as in the benzoylation mentioned above. The N^{α}-methyl form of Boc-Arg or the derivative thereof produced by this reaction is then treated with p-toluenesulfonyl chloride to tosylate the guanidino group in the side chain in accordance with a known method (J. Ramachandran, C.H. Li, J. Org. Chem., 27, 4006 (1962)). The peptide can be produced by using this derivative according to the known, so-called solid phase synthesis method for peptide (awarded the Nobel Prize in Chemistry) (R.B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963)).

[0053]

Hereinafter, the method for introducing a substituent into the carboxyl group for R^5 will be described briefly. For example, if it is desired to introduce an alkyl group (e.g., methyl group, ethyl group) or a benzyl group into the carboxyl group for R^5, esterification of Arg or a derivative thereof is performed in accordance with a known method (H. Yajima, Y. Kiso, K. Kitagawa, Chem. Pharm. Bull., 22, 1079 (1974) and M. Brenner, W. Huber, Helv. Chim. Acta, 36, 1109 (1953)). The material thus produced may be used as a starting material and subjected to reaction (e.g., benzoylation) in the same manner as in the benzoylation reaction or the like described above. In this manner, various compounds can be synthesized.

[0054]

Hereinafter, the method for synthesis of a compound of the general formula (I) wherein R^5 is $-COO-[NR^{54}-CHR^{53}-CO-(NH-CHR^{52}CO-)_p]$ will be described briefly. If R^{54} is a hydrogen atom, a protected amino acid resin having a C-terminal amino acid bound to Merrifield resin (polystyrene

resin) is prepared according to the Gisin method (B.F. Gisin, Helv. Chem.Acta, 56, 1476 (1973)). Using the protected amino acid resin as a starting material, the peptide solid-phase synthesis (R.B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963)) is repeated p-1 times and Boc-NR^{54}-CHR^{53}-COOH is then condensed. In the next step, Boc-Arg(Tos) (Peptide Institute, Inc., Minoo-shi, Osaka, Japan) or an Arg derivative which has been treated with p-toluenesulfonyl chloride to tosylate the guanidino group in the side chain (J. Ramachandran, C.H. Li, J. Org. Chem., 27, 4006 (1962)) is further bound by the peptide solid phase synthesis method. The resulting product is treated with hydrogen fluoride (HF) (S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, H. Sugihara, Bull. Chem. Soc. Jpn, 40, 2164 (1967)) to produce the desired product.

[0055]

A compound of the general formula (I) wherein R^5 is $-COO-[NR^{54}-CHR^{53}-CO-(NH-CHR^{52}CO-)_p]$ where R^{54} is a methyl group, can be produced as follows: N-Me-Arg(mono-methyl,Tos), N-Me-ADMA(Tos) or N-Me-SDMA(Tos) described above is butyloxycarbonylated to produce Boc-N-Me-Arg(mono-methyl,Tos), Boc-N-Me-ADMA(Tos) or Boc-N-Me-SDMA(Tos), respectively, which is then introduced into a desired site by the peptide solid phase synthesis method described above to prepare the desired product.

[0056]

If the compound of the general formula (I) has an acidic functional group (e.g., a carboxyl group), it may be provided in the form of a salt with a base (e.g., a pharmaceutically acceptable base) in a conventional manner. Example of such include salts with sodium, potassium, aluminum and calcium. If the compound of the general formula (I) has a basic functional group (e.g., an amino group, a mono-substituted amino group), it may be provided

in the form of a salt with an acid (e.g., a pharmaceutically acceptable acid) in a conventional manner. Examples of such salt include a hydrochloride, a sulfate, an acetate and a fumarate.

[0057]

The compound of the general formula (I) or a salt thereof can be used as a peptidylarginine deiminase V inhibitor.

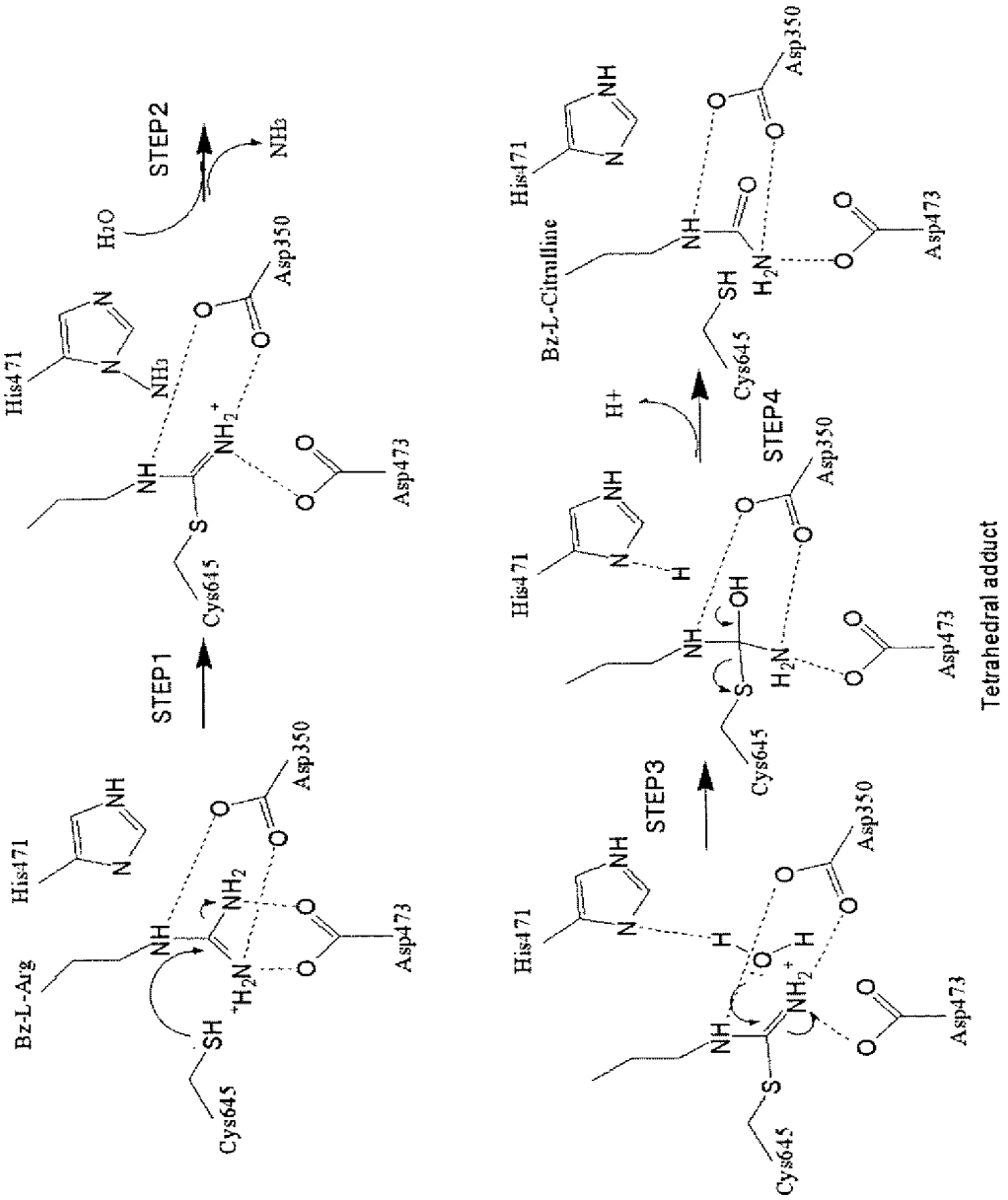
[0058]

2. Peptidylarginine deiminase V (PAD V) inhibitor

The present invention provides a peptidylarginine deiminase V inhibitor comprising as the active ingredient a substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism as shown in the following scheme between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine.

[0059]

[Formula 19]



In the scheme, Asp350, His471, Asp473 and Cys645 represent an aspartic acid residue at position 350, a histidine residue at

position 471, an aspartic acid residue at position 473 and a cysteine residue at position 645, respectively, in the amino acid sequence depicted in SEQ ID NO:1.

[0060]

The substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine may be an arginine derivative. The arginine derivative may be such that each of the amino and guanidino groups in arginine has a substituent while the carboxyl group in arginine optionally has a substituent. Specifically, the arginine derivative is a compound represented by the general formula (I) or a salt thereof.

[0061]

The substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine can be examined utilizing all or part of the three-dimensional structural coordinates of peptidylarginine deiminase V or its protein mutants thereof. For example, a substance which can be recognized by peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 is examined (e.g., identified, searched, evaluated or designed) on a computer system utilizing all or part of the three-dimensional structural coordinates of Ca^{2+}-free PAD V disclosed in Japanese Patent Application No. 2003-358459 or all or part of coordinates where the root mean square deviations thereof for bond length and bond angle are 0.019 angstrom and 1.887°, respectively; or all or part of the three-dimensional structural coordinates of a PAD V-calcium ion-substrate complex disclosed in Japanese Patent Application

No. 2003-358459 or all or part of coordinates where the root mean square deviations thereof for bond length and bond angle are 0.017 angstrom and 1.839°, respectively. Next, the substance is added with or substituted by an appropriate atom or atomic group at a proper position in the substance. In this manner, a substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between a substance recognized by peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine can be designed. The computer system to be used in the examination of the substance is not particularly limited, and any system may be used as long as a program for the examination of the substance can be run on it. Exemplary programs include DOCK (Science, 1992, 257, 1078), Gold4, Glide, FlexX (J. Mol. Biol., 1996, 261, 470), AutoDock (J. Comput. Chem., 1998, 19, 1639), ICM (J. Comput. Chem., 1994, 15, 488), and Ludi.

[0062]

If it is desired to design a substance capable of inhibiting any one or all of the steps 1 to 4, it is preferred that the hydrogen atom on the group $=NH_2(+)$ in arginine and/or the hydrogen atom on the group $-NH_2$ in arginine are/is substituted by an alkyl group (e.g., methyl group, ethyl group) and/or -NH be substituted by $-CH^2-$.

[0063]

The substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine may be a naturally occurring or synthetic product, and it may be a polymeric or low-molecular compound.

[0064]

The substance capable of inhibiting any one of the steps

1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine can be produced by any of the known procedures depending on the types of the substance.

[0065]

Next, the interaction of the substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine exhibits with respect to peptidylarginine deiminase V (e.g., dissociation constant with respect to peptidylarginine deiminase V), as well as the enzymatic activity of peptidylarginine deiminase V in the presence of the substance capable of inhibiting any one of the steps 1 to 4 in the reaction mechanism between peptidylarginine deiminase V having the amino acid sequence depicted in SEQ ID NO:1 and benzyl-L-arginine may be determined. The dissociation constant with respect to peptidylarginine deiminase V can be measured by performing a surface plasmon resonance experiment using BIACORE3000 (Pharamacia Biosensor AB). Briefly, peptidylarginine deiminase V is immobilized on the surface of a sensor chip, a substance to be tested is poured onto the sensor chip and, when the reaction system reaches an equilibrium, the dissociation constant is measured by the Schatchard plot analysis. The enzymatic activity of peptidylarginine deiminase V can be measured in accordance with the method described in Nakashima, K., Hagiwara, T., Ishigami, A., Nagata, S., Asaga, H., Kuramoto, M., Senshu, T. and Yamada, M. (1999) Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1α,25-dihydroxyvitamin D_3. J. Biol. Chem., 274, 27786-27792. A substance capable of decreasing the enzymatic

activity of peptidylarginine deiminase V can be used as a peptidylarginine deiminase V inhibitor.

[0066]

The peptidylarginine deiminase V inhibitor of the present invention may be administered to a human or other animals in the form of a pharmaceutical preparation or it may be used as a reagent for experimental purposes. The peptidylarginine deiminase V inhibitor of the present invention may be used singly or in combination with other therapeutic agents (e.g., other prophylactic/therapeutic agents for rheumatoid arthritis).

[0067]

When the peptidylarginine deiminase V inhibitor of the present invention is administered to a human, the inhibitor can be administered orally at about 0.1 to 9000 mg/kg body weight per day, preferably about 1 to 900 mg/kg body weight per day, in terms of the amount of the active ingredient, either as a single dose or in divided portions. However, the dose or the frequency of administration may vary as required, depending on the conditions or age of the patient, route of administration or the like.

[0068]

The peptidylarginine deiminase V inhibitor of the present invention may be administered orally in the form of such preparations as tablet, capsule, granule, powder or syrup, or it may be administered parenterally in the form of such preparations as an injectable solution or suppository through intraperitoneal or intravenous injection. The content of the active ingredient in the preparation may vary within the range from 1 to 90% by weight. For example, when administered in the form of such preparations as tablet, capsule, granule or powder, the active ingredient is preferably contained in the preparation at a concentration of 5

to 80% by weight; when administered in the form of a liquid preparation such as a syrup, the active ingredient is preferably contained in the preparation at a concentration of 1 to 30% by weight; and when administered parenterally in the form of an injectable solution, the active ingredient is preferably contained in the solution at a concentration of 1 to 10% by weight.
[0069]

The peptidylarginine deiminase V inhibitor of the present invention can be formulated into a pharmaceutical preparation in a conventional manner using pharmaceutical additives such as: excipients (e.g., saccharides including lactose, saccharose, glucose and mannitol; starches including potato, wheat and corn starches; inorganic substances including calcium carbonate, calcium sulfate and sodium hydrogen-carbonate; crystalline cellulose); binders (e.g., starch gel, gum arabic, gelatin, sodium alginate, methylcellulose, ethylcellulose, polyvinyl pyrrolidone, polyvinyl alcohol, hydroxylpropylcellulose, carmelose); lubricants (e.g., magnesium stearate, talc, hydrogenated vegetable oils, macrogol, silicone oil); disintegrants (e.g., starch, agar, gelatin powder, crystalline cellulose, CMC·Na, CMC·Ca, calcium carbonate, sodium hydrogen-carbonate, sodium alginate); flavoring agents (e.g., lactose, saccharose, glucose, mannitol, aromatic essential oils); solvents (e.g., water for injection, sterile purified water, sesame oil, soybean oil, corn oil, olive oil, cottonseed oil); stabilizers (e.g., inert gases including nitrogen and carbon dioxide; chelating agents including EDTA and thioglycolic acid; reducing agents including sodium hydrogen-sulfite, sodium thiosulfate, L-ascorbic acid and rongalit); preservatives (e.g., paraoxybenzoic acid ester, chlorobutanol, benzyl alcohol, phenol,

benzalkonium chloride); surfactants (e.g., hydrogenated castor oil, polysorbate 80, polysorbate 20); buffering agents (e.g., sodium citrate, acetate or phosphate, boric acid); and diluents.

[0070]

The peptidylarginine deiminase V inhibitor of the present invention can be used for the prevention and/or treatment of diseases associated with peptidylarginine deiminase V (e.g., rheumatoid arthritis). The peptidylarginine deiminase V inhibitor of the present can also be used in the study of peptidylarginine deiminase V.

[Effect of the Invention]

[0071]

According to the present invention, peptidylarginine deiminase V inhibitors are provided. The inhibitors can be used for the prevention and/or treatment of diseases associated with peptidylarginine deiminase V (e.g., rheumatoid arthritis).

[Best Mode for Carrying out the Invention]

[0072]

Hereinbelow, the present invention will be described in great detail with reference to the following examples. Note that the examples are for illustrative purposes only and the scope of the invention is not limited to these examples.

[Examples]

[0073]

[Production Example] Synthesis of Bz-Arg derivatives

Each of Arg derivatives (Arg: Nacalai Tesque Inc., Kyoto, Japan; citrulline: Sigma, St louis, USA;

N^G-monomethyl-L-arginine: Wako Pure Chemical Industries, Ltd., Osaka, Japan; ADMA (N^G,N^G-dimethyl-L-argnine): ALEXIS Biochemicals, Lausen, Switzerland; and SDMA (N^G,$N^{G'}$-dimethyl-L-argnine): ALEXIS Biochemicals, Lausen, Switzerland) (10 μmol) was dissolved in 0.1 M $NaHCO_3$ (200 μl), and Bz_2O(10 μmol)/DMF(200 μl) was added to the solution. After stirring, the mixture was allowed to stand at room temperature for 1 hour. The reaction solution was diluted with water (200 μl), and then washed with ethyl acetate (500 μl) three times. The resulting aqueous solution was added with 6 M HCl (100 μl) and then washed with ethyl acetate (500 μl) four times. The resulting reaction solution was subjected to reverse-phase HPLV to purify the desired Bz-Arg derivative (1: Bz-Arg, 2: Bz-Arg (mono-methyl), 3: Bz-ADMA, 4: Bz-SDMA). After the purification, all of the Bz-Arg derivatives were obtained at yields of around 40%.

Conditions for HPLC
Waters M600 multi-solvent delivery system
UV: 220 nm
Column: Develosil ODS-UG-5 (4.6 x 150 mm)
Temp.: 30°C
Solvent: Starting from 5% acetonitrile in a 0.05% aqueous TFA solution, the concentration of acetonitrile was increased at a rate of 1%/min.

[0074]

The HPLC charts of the final purified products are shown in Fig. 2, wherein the reference number 1 represents a peak of Bz-Arg, 2 for a peak of Bz-Arg (mono-methyl), 3 for a peak of

Bz-ADMA, and 4 for a peak of Bz-SDMA.

[0075]

The individual compounds were identified by MALDI-TOF MS (mass spectrometry).

Apparatus: Applied Biosystems Voyager System 6178

[0076]

[Table 1]

Atoms	Accurate mass number
C	12
H	1.00783
N	14.0031
O	15.9949

		MALDI-TOF Mass	
	Accurate mass number (M)	Calculated M+H	Found M+H
Bz-Arg	278.1	279.1	279.5
Bz-Arg (mono-methyl)	292.2	293.2	293.6
Bz-ADMA	306.2	307.2	307.6
Bz-SDMA	306.2	307.2	307.6
Bz-citrulline	279.1	280.1	280.3

[0077]

[Test Example] Inhibition reaction of Bz-Arg derivatives on PAD V digestion

A buffer solution B (0.1 M Tris/HCl, 10 mM $CaCl_2$, 2 mM DTT, pH 7.6, 125 µl), a Bz-Arg derivative (0.1 M Tris/HCl, 10 mM $CaCl_2$, pH 7.6, 25 µl (a solution prepared in a concentration of 1 nmol/µl)) and PAD V (1 µl) were mixed together under ice-cooling to give a Bz-Arg solution. Twenty µl each of Bz-Arg (mono-methyl),

Bz-ADMA, Bz-SDMA and a buffer solution A (0.1 M Tris/HCl, 10 mM $CaCl_2$, pH 7.6) (a solution prepared in a concentration of 1 nmol/μl) was mixed with the Bz-Arg solution (30 μl) and allowed to react at 37°C for 40 or 60 minutes. The reaction was quenched with 1 M HCl (50 μl) and then subjected to reverse-phase HPLC to separate the reaction mixture. As a result, Bz-ADMA was found to show the most potent inhibitory effect, followed by Bz-Arg (mono-methyl). Bz-SDMA showed no inhibitory effect at the concentration employed in the test. The results as determined 40 minutes and 60 minutes after the start of reaction are shown in Fig. 3 and Fig. 4, respectively. In Figs. 3 and 4, the reference number 1 represents the result with no inhibitor, 2 for the result with Bz-Arg (mono-methyl), 3 for the results with Bz-ADMA, and 4 for the result with Bz-SDMA; the vertical axis indicates the sample number and the horizontal axis indicates the yield of the deimination reaction (i.e., yield of the Bz-citrulline produced).

[Brief Description of Drawings]

[0078]

Fig. 1 shows the schematic illustration of the reaction mechanism for deimination of PAD V as proposed by the present inventors.

Fig. 2 shows the HPLC charts of final purified products produced in the Production Example, in which the reference number 1 represents a peak of Bz-Arg, 2 for a peak of Bz-Arg (mono-methyl), 3 for a peak of Bz-ADMA, and 4 for a peak of Bz-SDMA.

Fig. 3 shows the results of an inhibition reaction on the PAD V digestion of the Bz-Arg derivatives produced in the Production Example (as determined 40 minutes after the reaction was initiated).

Fig. 4 shows the results of an inhibition reaction on the PAD V digestion of the Bz-Arg derivatives produced in the Production Example (as determined 60 minutes after the reaction was initiated).

[Free Text of Sequence Listing]

[0079]

SEQ ID NO:1 shows the amino acid sequence of human peptidylarginine deiminase V.

[SEQUENCE LISTING]

SEQUENCE LISTING

<110> Sato, Mamoru

<120> Peptidylarginine Deiminase V inhibitors

<130> P03-073

<140>
<141>

<160> 1

<170> PatentIn Ver. 2.1

<210> 1
<211> 663
<212> PRT
<213> Homo sapiens

<400> 1

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 1               5                  10                  15

Ala Val Cys Val Leu Gly Thr Leu Thr Gln Leu Asp Ile Cys Ser Ser
            20                  25                  30
```

Ala Pro Glu Asp Cys Thr Ser Phe Ser Ile Asn Ala Ser Pro Gly Val
35 40 45

Val Val Asp Ile Ala His Ser Pro Pro Ala Lys Lys Lys Ser Thr Gly
50 55 60

Ser Ser Thr Trp Pro Leu Asp Pro Gly Val Glu Val Thr Leu Thr Met
65 70 75 80

Lys Ala Ala Ser Gly Ser Thr Gly Asp Gln Lys Val Gln Ile Ser Tyr
85 90 95

Tyr Gly Pro Lys Thr Pro Pro Val Lys Ala Leu Leu Tyr Leu Thr Ala
100 105 110

Val Glu Ile Ser Leu Cys Ala Asp Ile Thr Arg Thr Gly Lys Val Lys
115 120 125

Pro Thr Arg Ala Val Lys Asp Gln Arg Thr Trp Thr Trp Gly Pro Cys
130 135 140

Gly Gln Gly Ala Ile Leu Leu Val Asn Cys Asp Arg Asp Asn Leu Glu
145 150 155 160

Ser Ser Ala Met Asp Cys Glu Asp Asp Glu Val Leu Asp Ser Glu Asp
165 170 175

Leu Gln Asp Met Ser Leu Met Thr Leu Ser Thr Lys Thr Pro Lys Asp
180 185 190

Phe Phe Thr Asn His Thr Leu Val Leu His Val Ala Arg Ser Glu Met
195 200 205

Asp Lys Val Arg Val Phe Gln Ala Thr Arg Gly Lys Leu Ser Ser Lys
210 215 220

Cys Ser Val Val Leu Gly Pro Lys Trp Pro Ser His Tyr Leu Met Val
225 230 235 240

Pro Gly Gly Lys His Asn Met Asp Phe Tyr Val Glu Ala Leu Ala Phe
245 250 255

Pro Asp Thr Asp Phe Pro Gly Leu Ile Thr Leu Thr Ile Ser Leu Leu
260 265 270

Asp Thr Ser Asn Leu Glu Leu Pro Glu Ala Val Val Phe Gln Asp Ser
275 280 285

Val Val Phe Arg Val Ala Pro Trp Ile Met Thr Pro Asn Thr Gln Pro
290 295 300

Pro Gln Glu Val Tyr Ala Cys Ser Ile Phe Glu Asn Glu Asp Phe Leu
305 310 315 320

Lys Ser Val Thr Thr Leu Ala Met Lys Ala Lys Cys Lys Leu Thr Ile
325 330 335

Cys Pro Glu Glu Glu Asn Met Asp Asp Gln Trp Met Gln Asp Glu Met
340 345 350

Glu Ile Gly Tyr Ile Gln Ala Pro His Lys Thr Leu Pro Val Val Phe
355 360 365

Asp Ser Pro Arg Asn Arg Gly Leu Lys Glu Phe Pro Ile Lys Arg Val
370 375 380

Met Gly Pro Asp Phe Gly Tyr Val Thr Arg Gly Pro Gln Thr Gly Gly
385 390 395 400

Ile Ser Gly Leu Asp Ser Phe Gly Asn Leu Glu Val Ser Pro Pro Val
405 410 415

Thr Val Arg Gly Lys Glu Tyr Pro Leu Gly Arg Ile Leu Phe Gly Asp
420 425 430

Ser Cys Tyr Pro Ser Asn Asp Ser Arg Gln Met His Gln Ala Leu Gln
435 440 445

Asp Phe Leu Ser Ala Gln Gln Val Gln Ala Pro Val Lys Leu Tyr Ser
450 455 460

Asp Trp Leu Ser Val Gly His Val Asp Glu Phe Leu Ser Phe Val Pro
465 470 475 480

Ala Pro Asp Arg Lys Gly Phe Arg Leu Leu Leu Ala Ser Pro Arg Ser
485 490 495

Cys Tyr Lys Leu Phe Gln Glu Gln Gln Asn Glu Gly His Gly Glu Ala
500 505 510

Leu Leu Phe Glu Gly Ile Lys Lys Lys Lys Gln Gln Lys Ile Lys Asn
515 520 525

Ile Leu Ser Asn Lys Thr Leu Arg Glu His Asn Ser Phe Val Glu Arg
530 535 540

Cys Ile Asp Trp Asn Arg Glu Leu Leu Lys Arg Glu Leu Gly Leu Ala
545 550 555 560

Glu Ser Asp Ile Ile Asp Ile Pro Gln Leu Phe Lys Leu Lys Glu Phe
565 570 575

Ser Lys Ala Glu Ala Phe Phe Pro Asn Met Val Asn Met Leu Val Leu
580 585 590

Gly Lys His Leu Gly Ile Pro Lys Pro Phe Gly Pro Val Ile Asn Gly
595 600 605

Arg Cys Cys Leu Glu Glu Lys Val Cys Ser Leu Leu Glu Pro Leu Gly
610 615 620

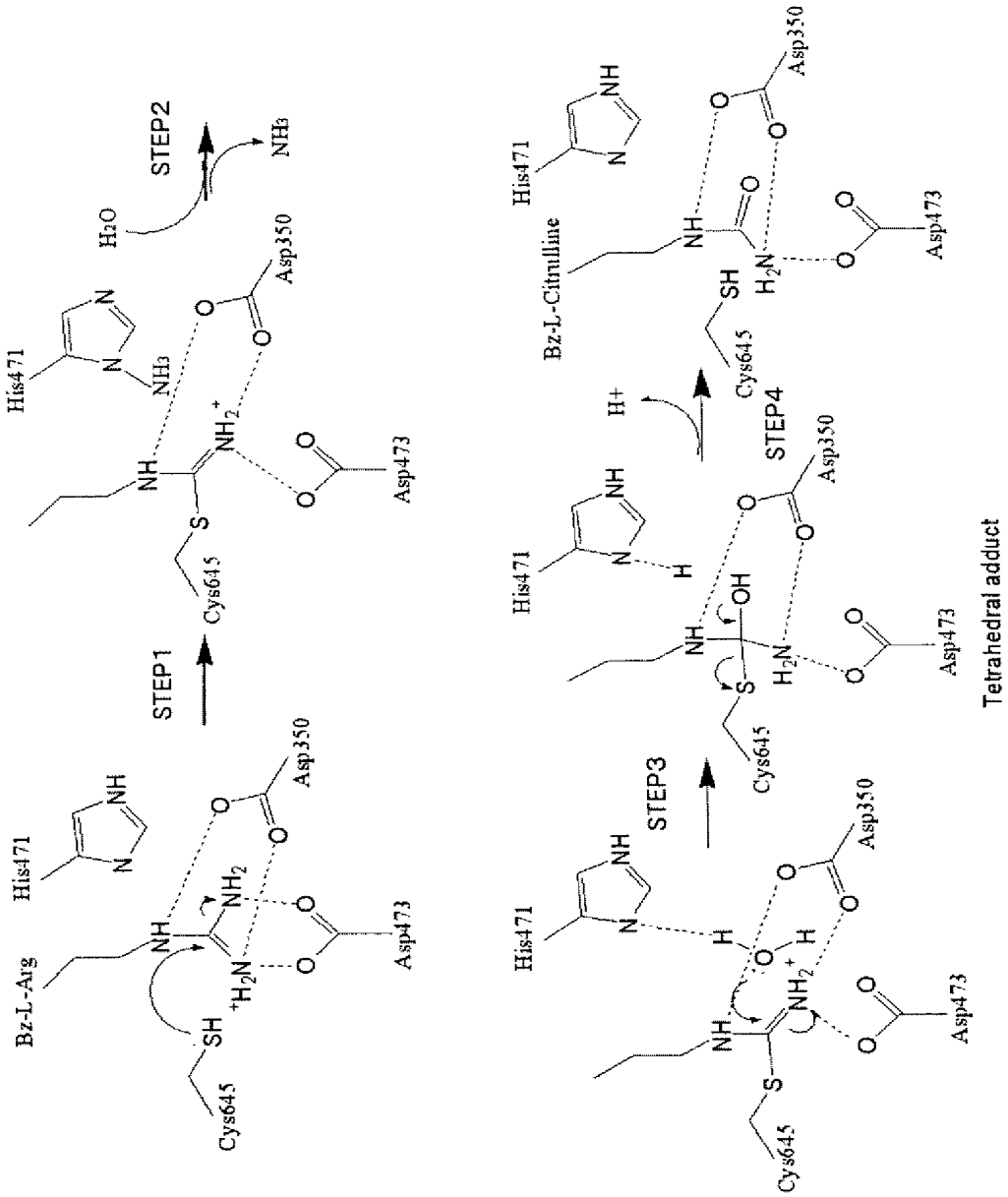
Leu Gln Cys Thr Phe Ile Asn Asp Phe Phe Thr Tyr His Ile Arg His
625 630 635 640

Gly Glu Val His Cys Gly Thr Asn Val Arg Arg Lys Pro Phe Ser Phe
645 650 655

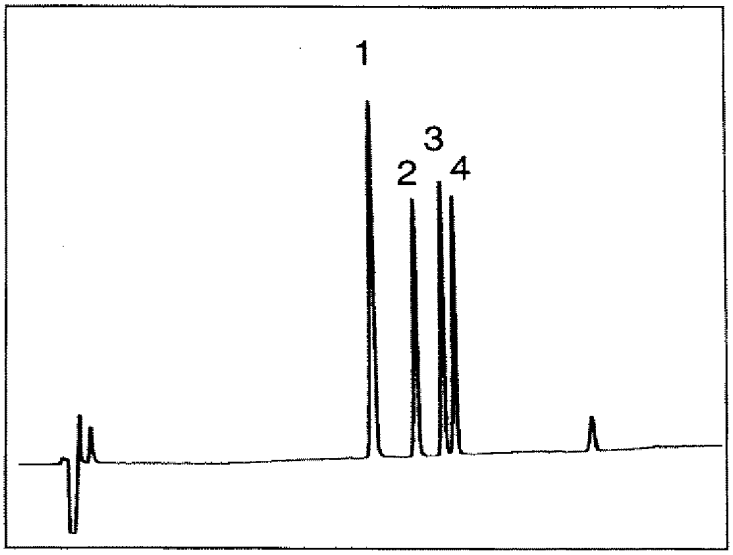
Lys Trp Trp Asn Met Val Pro
660

[Name of Document] Drawing

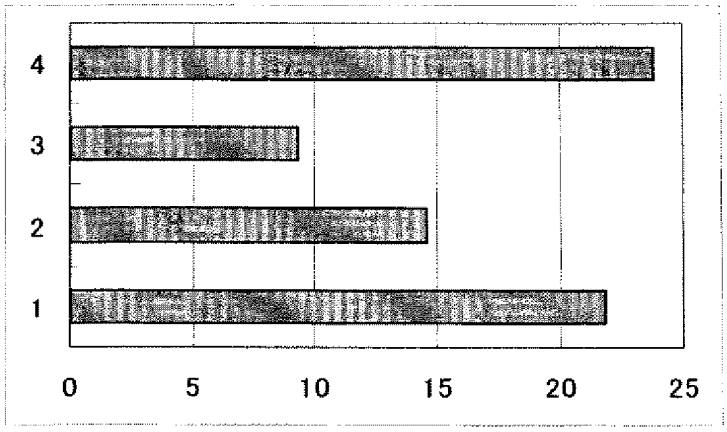
[Figure 1]



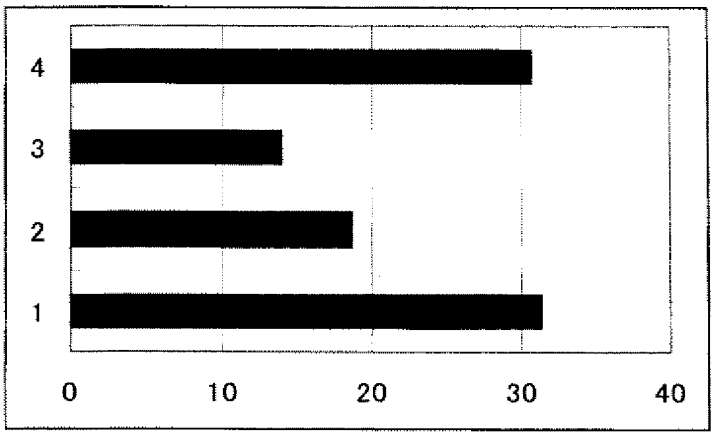
[Figure 2]



[Figure 3]



[Figure 4]



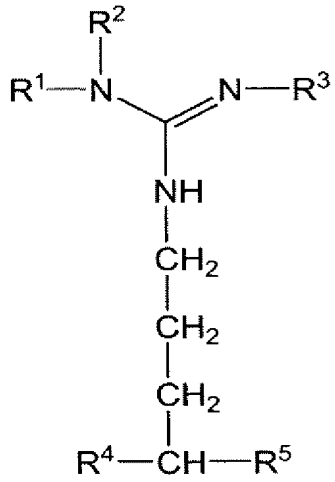
[Name of Document] Abstract

[Abstract]

[Object] The object of the present invention is to design a novel substance capable of inhibiting the enzymatic activity of PAD V and to develop a new drug against rheumatoid arthritis.

[Means of Solution] A compound represented by the general formula (I) or a salt thereof is provided:

[Formula 1]



(I)

wherein R^1, R^2 and R^3 each independently represent a hydrogen atom or an alkyl group having 1 to 3 carbon atoms, provided that at least one of R^1, R^2 and R^3 does not represent a hydrogen atom; R^4 represents an amino group which has a substituent; and R^5 represents a carboxyl group which may have a substituent.

[Selected Figure] Figure 1

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[Filing Date] March 26, 2004

[Addressee] Commissioner of the Patent Office

[Identification of the Case]

[Application Number] Japanese Patent Application No. 2004-28467

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[Identification Number] 100107870

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[Name or Appellation] Kenichi NOMURA

[Indication of Fees]

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